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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/558 — A1

(11) International Publication Number: WO 94/23299

(43) International Publication Date: 13 October 1994 (13.10.94)

(21) International Application Number: PCT/US94/03488

(22) International Filing Date: 30 March 1994 (30.03.94)

(30) Priority Data:
08/040,557 31 March 1993 (31.03.93) US

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(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: MULTIPLE ASSAY DEVICE

(57) Abstract

The present invention provides devices and methods for simultaneous detection of multiple analytes in a biological sample. Also provided are devices and methods for detecting an analyte in a series of samples. Using the present invention the analyte assays are easy to perform and inexpensive, providing a means for analyte detection or quantitation in the home or health care provider's office.

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MULTIPLE ASSAY DEVICE

BACKGROUND OF THE INVENTION

The present invention relates generally to devices and methods for detection of analytes in biological samples. More specifically, the present invention provides devices and methods for efficient and relatively inexpensive simultaneous detection of several analytes in a single sample or the serial detection of one or more analytes in several samples.

Detection of analytes in biological samples has become a vital tool of medical diagnostics. Often it is desirable to detect many different analytes in a single biological sample. For example, screening for exposure to certain diseases that have common modes of transmission is useful in many patient populations, e.g., public health screening for sexually transmitted diseases. Prenatal screening of pregnant women for infections associated with congenital malformations is another common instance in which simultaneous detection of different analytes is desirable. Previously, it was generally necessary to send biological samples to a reference laboratory for analysis. While reference laboratories could simultaneously measure some combinations of different analytes, several useful combinations required separate assays or expensive instrumentation.

Also, it is often clinically useful to detect the presence or amount of one or more analytes in a biological sample on several occasions. Serial determinations of disease associated analytes can provide an indication of the efficacy of treatment. For example, serial determinations of glucose provide a guide for insulin therapy of diabetes. In another context, serial determinations of certain hormones can identify periods of fertility or infertility during a woman's menstrual cycle and provide guidance for facilitating or avoiding conception. Performance of these hormonal assays in

a laboratory is impractical because submitting daily samples is expensive, inconvenient, and intrusive for patients.

Background Art

Several devices have been developed for detection of analytes in biological samples. Among these, devices that do not require extensive training or instrumentation are the most relevant to the present invention.

Immunoassays have been developed for home use. For example, the OvuQuick Self-Test for ovulation prediction produced by Monoclonal Antibodies, Inc. is a home test kit for measurement of luteinizing hormone. The kit contains a multi-step assay requiring mixing of reagents and multiple sample treatment steps. The kit contains several different assay test devices which only provide one assay and requires refrigeration between uses. Other multi-step immunoassays have also been described, e.g., U.S. Patent No. 4,632,901 of Valkirs et al.

Devices for the detection of analytes in biological samples have been developed which contain premixed reagents. International Patent Publication WO86/06488 of Barnett describes a device that containing reservoirs of reagents. Following introduction of the sample, to the device the reservoirs may be ruptured to mix the reagents with the sample. While this alleviates the need for the user to prepare and add reagents to the sample, the reservoirs may prematurely rupture destroying the utility of the assay device and potentially contaminating surrounding articles.

Lateral flow assays for one step detection of analytes in biological samples have also been developed. U.S. Patent No. 4,943,522 describes lateral flow assays for the detection of a variety of analytes. Devices are described that provide a one step means of detecting the presence or concentration of a single analyte in a single sample.

U.S. Patent Nos. 4,938,927 and 5,141,875 describe devices and methods for rotary fluid assays. Fluid samples are applied to bodies that have wicking characteristics and

react with reagents present on the bodies. The bodies are described for use with laboratory centrifuges. The devices include means for occluding fluid flow between different segments of the devices so that different assays may be performed.

Devices and methods are needed in the art which will provide simple and convenient simultaneous detection of multiple analytes in biological samples. Desirably, the devices and methods are completely self contained and do not require additional instrumentation. The devices should be economical to produce and simply assembled. Also desirable are devices and methods for serial determinations of one or more analytes in biological samples which are simple and accurate and can be performed in the physician's office or even by patients in the privacy of their home. Quite surprisingly, the present invention fulfills these and other needs.

SUMMARY OF THE INVENTION

The present invention provides devices for simultaneous detection of a plurality of target analytes in a sample, having a first matrix defining a flow path; a first sample receiving zone on the first matrix; and a plurality of assay strips located downstream from the first sample receiving zone, wherein each assay strip detects a different target analyte, which assay strips each comprise a labelling zone having a means for specifically labelling the target analyte detected by the assay strip and a capture zone located downstream from the labelling zone; whereby application of the sample to the first sample receiving zone results in specific binding of the target analytes in the sample to labelling means on the assay strips and accumulation of labelled target analytes in the capture zones.

The devices of the present invention may further include positive and negative test strips. The test strips serve as internal positive and negative controls for the assay devices. Additionally, the assay strips may contain positive

procedural control lines that provide protection against false negative results due to device malfunctions.

In another embodiment of the present invention devices are provided for a plurality of determinations of the presence or amount of an analyte in a sample. The devices contain: a matrix defining a flow path; a plurality of sample receiving zones on the matrix; a plurality of assay strips located downstream from the sample receiving zones, wherein each assay strip is in fluid connection with only one sample receiving zone and comprises a labelling zone having a means for labelling the analyte and a capture zone located downstream from the labelling zone; whereby application of the sample to the sample receiving zone results in specific binding of the analyte in the sample to the labelling means on the assay strips and accumulation of labelled analyte in the capture zones. Devices of this type may also contain test strips for internal control purposes.

Methods for simultaneously determining the presence or amount of a plurality of analytes in a sample, comprising: applying the sample to a device of the present invention that detects multiple analytes in a sample; and observing the accumulation of visible label within the capture zone of the device as a result of analyte present in the sample specifically binding to the labelling complex in the labelling means and the resulting analyte-labelling complexes flowing into and being captured within the capture zone. Methods for serially detecting the presence and/or amount of an analyte in a series of samples are also provided. The samples are applied to a device of the present invention for serially detecting an analyte in multiple samples and observing accumulation of label in a capture zone of the device following each test. The methods may also include steps for assessing the accuracy of the results by checking positive and negative controls. Kits for convenient practice of the invention are also described.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates one type of assay strip employed in devices constructed in accordance with the principles of the present invention.

Fig. 2 illustrates an assay strip having a positive procedural control line.

Fig. 3 illustrates a matrix of a device for simultaneous detection of different analytes in a sample constructed in accordance with the principles of the present invention.

Fig. 4 illustrates a top plate for a device for the simultaneous detection of different analytes in a biological sample.

Fig. 5 illustrates a matrix of a device for serial detection of an analyte in different biological samples constructed in accordance with the principles of the present invention.

Fig. 6 illustrates a top plate for a device for serial detection of an analyte in different biological samples.

Fig. 7 illustrates a matrix of a device for the simultaneous detection of different analytes in a sample having positive and negative control strips.

Fig. 8 illustrates a top plate for a device for the simultaneous detection of different analytes in a sample having positive and negative control strips.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides devices and methods for one-step detection of analytes in biological samples. The devices and methods provide a simple and rapid method of detection that may be performed by medical assistants in the office of a health care provider or even by the patient in the privacy of the home. The assays of the present invention are self contained and require no special instrumentation. The devices are also easy and economical to produce, providing greater availability to the public. The quickness with which

assay results may be obtained also allows for prompt treatment or other actions, as appropriate.

Several different analytes in a biological sample may be simultaneously detected by devices of the present invention. Devices of this type are particularly useful for detection of analytes such as antibodies in biological samples. Antibodies to different infectious agents which are transmitted in similar manners, e.g., sexually transmitted diseases may be quickly identified by simple screening in sexually transmitted disease clinics. Antibodies to infectious agents that cause diseases in particular patient populations, e.g., the pathogens causing congenital malformations in TORCHES syndrome during pregnancy, may also be simultaneously detected with the devices of the present invention. IgE antibodies to different common allergens may be detected as taught by the present invention to diagnose or confirm atopic conditions. Persons of skill in the art will appreciate that other analytes may also be conveniently detected by devices and methods of the present invention.

Alternatively, devices of the present invention may detect a single analyte in different samples. Devices of this type are particularly useful in serial determinations of an analyte that reflects a physiological state or disease condition. For example, daily serial determinations of urinary luteinizing hormone and progesterone metabolite levels can indicate infertile periods during a woman's menstrual cycle and provide an easy in home method of facilitating contraception by the rhythm method.

The devices and methods of the present invention employ lateral flow methodology for detection of analytes. The samples are generally biological fluids. The biological fluid sample may be whole blood, plasma, serum, nasal secretions, sputum, salvia, urine, sweat, transdermal exudates, cerebrospinal fluid, or the like. Analytes in biological tissue samples may also be detected by homogenizing or otherwise liquefying the tissue sample. The sample, whether a fluid or liquified tissue, may be diluted with

physiological buffers, such as physiological saline, to facilitate sample flow in the devices of the present invention. Persons of skill will appreciate that dilution of the sample will alter the concentration of analyte in the diluted sample and appropriate calculations will be required to normalize the assay results in some instances. The present invention may also be employed for the detection of analytes in samples which are not biological. For example, water samples may be conveniently analyzed for the presence of a variety of different toxins using devices of the present invention.

The devices and methods of the present invention employ lateral flow assay techniques as generally described in U.S. Patent Nos. 4,943,522; 4,861,711; 4,857,453; 4,855,240; 4,775,636; 4,703,017; 4,361,537; 4,235,601; 4,168,146; 4,094,647; co-pending application U.S.S.N. 07/639,967, European Patent Application Nos. 451,800; 158,746; 276,152; 306,772 and British Patent Application No. 2,204,398; each of which is incorporated herein by reference.

One embodiment of the present invention is a device for simultaneous detection of a plurality of target analytes in a sample. By "simultaneous detection of a plurality of analytes", it is meant that at least two analytes in a single sample may be detected by a single application of the sample to the device. The time required for detection of the different analytes will generally be approximately equal, although the times may vary significantly. So long as the detection of the different analytes is accomplished by a single application of sample to the device, the detection is considered simultaneous.

This embodiment of the present invention includes a first matrix defining a flow path; a sample receiving zone on the first matrix; and a plurality of assay strips located downstream from the sample receiving zone, wherein each assay strip detects a different target analyte, which assay strips each comprise a labelling zone having a means for specifically labelling the target analyte detected by the assay strip and a

capture zone located downstream from the labelling zone. Application of the sample to the sample receiving zone results in specific binding of the target analytes in the sample to labelling means on the assay strips and accumulation of labelled target analytes in the capture zones as the sample flows from sample receiving zone.

A variety of target analytes may be detected by the present invention. Antibodies to infectious agents may be detected. Antibodies to allergens may also be detected. IgE antibodies to specific allergens may be detected to diagnose or confirm allergic conditions. IgG antibodies to allergens may also be detected to assess the efficacy of desensitization treatments. Hormones, such as luteinizing hormone, progesterones, and progesterone metabolites may be measured to predict fertile periods in the menstrual cycle to assist either conception or contraception. Tumor related antigens, such as carcinoembryonic antigen, prostate specific antigen, and the like, may be detected by devices of the present invention as a screening tool for high risk populations. Persons of skill will appreciate other analytes which may be conveniently detected by the present invention.

The matrix of the devices of the present invention will typically be capable of non-bibulous lateral flow. By "non-bibulous lateral flow" is meant liquid flow in which all of the dissolved or dispersed components of the liquid are carried at substantially equal rates and with relatively unimpaired flow laterally through or across the matrix, as opposed to preferential retention of one or more components as would occur, e.g., in materials capable of adsorbing or imbibing one or more components.

A representative non-bibulous matrix material is high density polyethylene sheet material. Polyethylene sheets of this type are available from commercial sources,such as by Porex Technologies Corp. of Fairburn, Georgia, USA. Generally, the membrane has an open pore structure with a typical density, at 40% void volume, of 0.57 gm/cc and an average pore diameter of 1 to 250 micrometers, the average

generally being from 3 to 100 micrometers. Usually the membrane pore diameter is about 10 to about 50 μm, although other pore diameters may be employed for different purposes. For example, a membrane having large pores may filter particulates from a sample and selectively allow the fluid and dissolved compounds in the sample to flow downstream.

The membranes are from a few mils (.001 in) to several mils in thickness, typically in the range of 5 mils to 200 mils. The membrane may be backed by a generally water impervious layer, or may be totally free standing. Free standing matrixes will generally be thicker since structural support for the device will be provided by the matrix. Other non-bibulous membranes, such as polyvinyl chloride, polyvinyl acetate, copolymers of vinyl acetate and vinyl chloride, polyamide, polycarbonate, nylon, glass fiber, orlon, polyester polystyrene, and the like, or blends can also be used.

Bibulous materials, such as untreated paper, nitrocellulose, derivatized nylon, cellulose and the like may also be used following processing to provide non-bibulous flow. Bibulous materials may also act as filtering mechanisms as described above. Alternatively, blocking agents may block the forces which account for the bibulous nature of bibulous membranes. Suitable blocking agents include whole or derivatized bovine serum albumin or albumin from other animals, whole animal serum, casein, and non-fat dry milk.

The matrix defines a flow path for fluids applied to the matrix. The flow path is the natural movement of fluids placed on the matrix. The matrix has a sample receiving zone. The sample receiving zone is the portion of the matrix to which the samples are applied. Generally, the sample receiving zone will have a low analyte retention rate. Treatment of the sample receiving zone to immobilize a protein-blocking reagent on the surface, if necessary, will typically provide low retention properties. This treatment also provides increased wetability and wicking action to speed the downstream flow of the sample. The sample receiving zone

may also serve as a means for filtering particulates from the sample as described above.

The sample receiving zone is in fluid contact with at least two assay strips. All assay strips are located downstream of the sample receiving zone so that fluid from sample applied to the sample receiving zone will flow into each assay strip. In this embodiment of the present invention, each assay strip in a device will detect a different analyte. Thus, a number of analytes equal to the number of assay strips in the device will be simultaneously detected by this embodiment of the present invention.

The assay strips comprise a labelling zone and a capture zone. A means for labelling the target analyte is present in the labelling zone. The labelling means contain labelling complexes that specifically bind to target analyte in the patient sample. The labelling complexes are comprised of a visible label bound to a first analyte-binding substance. The first analyte-binding substance may be, e.g., an antibody or fragment thereof which specifically binds the analyte detected by the assay strip. Alternatively, the first analyte-binding substance may be an anti-ligand of a ligand analyte, e.g., a receptor for a particular hormone if the analyte detected by the assay strip is a hormone. If the analyte is an immunoglobulin, the first analyte-binding substance may be an antigen which is specifically bound by the immunoglobulin. The labelling complex is not immobilized to the labelling zone so that the sample may solubilize or otherwise remove the labelling complexes into the fluid of the sample.

A variety of visible labels may be bound to the first immunoglobulin-binding substance. The labels may be soluble or particulate and may include dyed immunoglobulin binding substances, simple dyes or dye polymers, dyed latex beads, dye-containing liposomes (such as described in U.S. Patent No. 4,695,554, incorporated herein by reference), dyed cells or organisms, or metallic, organic, inorganic, or dye sols. The labels may be bound to the analyte-binding

substance by a variety of means which are well known in the art such as described in U.S. Patent Nos. 4,863,875 and 4,373,932, each of which is incorporated herein by reference. Generally, the same label will be used on each assay strip employed in a device of the present invention.

Because the labelling complexes contain an analyte-binding substance and the labelling complexes are solubilized or dispersed into the patient sample, target analyte in the sample which reacts with the analyte-binding substance is contacted and bound by the labelling complexes prior to entering the capture zone. In this manner, the target analyte molecules are labelled. If any of the labelled target analyte molecules are retained in the capture zone of the device, the label provides a means for detection.

Each assay strip has a capture zone located downstream of the labelling zone. The capture zone typically contacts the labelling zone. A second analyte-binding substance is immobilized on the capture zone. If the analyte detected by the assay strip is an antigen-specific immunoglobulin, the second analyte-binding substance may be, e.g., an anti-immunoglobulin antibody or fragment thereof, or an antigen which is specifically bound by the immunoglobulin. The second analyte-binding substance may also be an anti-ligand of the target analyte as described above. As target analytes in the sample contact the capture zone, the analytes bind to the second analyte-binding substance and are retained in the capture zone.

Since target analytes present in the sample have been labelled by the labelling complexes, retention of target analytes in the capture zone is detected by observation of visible label accumulation. In some embodiments of the present invention the second analyte-binding substance will be immobilized to the capture zone in a specific pattern, such as a "+" or a "✓". The pattern can aid users in identifying positive results.

The accumulation of visible label may be assessed either visually or by optical detection devices, such as

reflectance analyzers, video image analyzers and the like. The accumulation of visible label can be assessed either to determine the presence or absence of label in the capture zone or the visible intensity of accumulated label which may by correlated with the concentration or titer (dilution) of target analytes in the patient sample. The correlation between the visible intensity of accumulated label and analyte concentration may be made by comparison of the visible intensity to a reference standard. Optical detection devices may be programmed to automatically perform this comparison by means similar to that used by the Quidel Reflective Analyzer, Catalog No. QU0801 (Quidel Corp., San Diego, CA). Visual comparison is also possible by visual evaluation of the intensity and a color key such as used in the Quidel Total IgE Test Catalog No. 0701 (a multi-step ELISA assay). Thus, target analyte concentration in the sample may be determined. When the sample has been diluted to reduce viscosity and facilitate flow, appropriate correction of the measure concentration will be required. Video analyzers may also be used to determine the concentration of target analyte in the sample from the intensity of the accumulated label.

The capture zone is often contacted by a bibulous absorbent zone. The absorbent zone is located downstream from the capture zone. The absorbent zone is a means for removing excess sample and unbound labelling complexes from the matrix of the device. Generally, the absorbent zone will consist of an absorbent material such as filter paper, a glass fiber filter, cellulose, or the like.

The devices of the present invention may also have a positive test strip and a negative test strip. The positive and negative test strips serve as internal controls to assess the functioning of the device. The test strips may be located on the matrix, downstream of and in fluid contact with the sample receiving zone. The positive test strip detects a non-target analyte that is typically present in all samples. Such non-target analytes may include, e.g., albumin or non-antigen specific IgG that is normally present in serum, plasma or

blood samples; urobilinogen in urine samples, and the like. The non-target analyte will vary according to the nature of the sample to be tested. The positive test strip contains a second labelling zone having a second means for labelling the non-target analyte, and a second capture zone. The second means for labelling the non-target analyte and the second capture zone are similar in character to the corresponding components of the assay strips as described above. The negative test strip generally contains a labelling zone having a label used in the assay strips. The label is not conjugated to an analyte-binding substance. The capture zone of the negative test strip may have a second analyte-binding substance. Alternatively, the capture zone may not have a second analyte-binding substance.

Application of the sample to the device results in fluid flow into the positive and negative test strips. On the positive test strip, the non-target analyte present in the sample will bind the means for labelling the non-target analyte which is then retained in the capture zone. A positive result is expected on the positive test strip if the device functions properly. Lack of a positive result indicates that a device malfunction has occurred and that the results on the assay strips are suspect. As the fluid flows onto the negative test strip, the unbound label is mixed with the fluid and carried to the capture zone. Because the label does not bind an analyte which is captured in the capture zone, no label should be retained and a negative result is expected. A positive result on the negative test strip indicates non-specific retention of the label in the capture and positive results on the assay strips are suspect.

In an alternative means for internal control testing, the device may have a second matrix and a second sample receiving zone. The positive and negative test strips are downstream of and in fluid contact with the second sample receiving zone. The construction of the positive test strip is similar to the positive test strips described above except that the labelling means includes a means for labelling a non-

target analyte which is present in a test fluid. The negative test strip is constructed as above, but lacks a bound label in the labelling zone.

A test fluid containing a known amount of the non-target analyte may be applied to the second sample receiving zone. The test fluid flows into the positive test strip and negative test strip. Because the positive test strip has a means for labelling the non-target analyte and may capture the labelled non-target analyte in the capture zone, a positive result is obtained if the device is functioning properly. Likewise, if the device functions properly, a negative result is expected on the negative test strip.

Devices of the present invention may also contain a procedural control line on each assay strip. The procedural control line is in the capture zone located downstream from the second analyte-binding substance. The procedural control line is a control binding substance which will specifically bind the labelling complexes of the labelling zone. As the labelling complexes, both bound to analyte and unbound, contact the procedural control line, the labelling complexes are captured and retained by the control binding substance. Thus, if the device functions properly and fluid reaches the procedural control line, label accumulates on the procedural control line ensuring sample contact with the second analyte-binding substance. Lack of label accumulation indicates device malfunction and assay results are suspect. Appropriate control binding substances will vary with the character of the labelling complexes. For example, if the first analyte-binding substance is an IgG antibody, the control binding substance may be an anti-IgG antibody, Staphylococcal Protein A or Protein G. Persons of skill will appreciate appropriate control binding substances.

The geometric configuration of the devices of the present invention is not critical and may vary. Generally, the assay strips, and positive and negative control strips, if present, extend radially from the sample receiving zone at equally spaced intervals. In a particularly convenient

embodiment, the device is fan shaped approximating a quarter circle. The number and dimensions of assay strips and control strips in each device may also vary depending on the use.

Analytes detected by devices of the present invention may vary considerably. One particularly useful embodiment for office prenatal screening by obstetricians or midwives employs assay strips which detect serum antibodies to toxoplasma gondii, rubella virus, human cytomegalovirus, herpes simplex virus, and treponema palladium organisms. Allergists may also find devices of the present invention particularly useful. The devices may be employed detect IgE to common antigens, such as mites, grasses, e.g. bermuda grass, timothy grass, bent grass, perennial rye grass, and the like; dander, such as dog dander, cat dander, and the like; foods, e.g., apples, shrimp, strawberries, shellfish, and the like; insect venom, such as mosquito venom, wasp venom, bee venom, and the like; trees, such as elm, oak, acacia, and the like; bacteria such as Streptococcus pneumonia, Helicobacter pylori and the like; viruses such as hepatitis virus, and the like; flowers, such as marigold, sunflower, and the like; and weeds, such as pigweed, ragweed, Russian thistle, and the like. This list is only partial and those of skill will readily appreciate that immunoglobulins to other allergens could be detected by the methods and devices of the present invention. Devices of the present invention may also be used to evaluate IgG levels to specific allergens to assess the efficacy of desensitization therapy.

Multiple hormones may also be measured by devices of the present invention. For example, pan-hypopituitarism may be detected by simultaneously assaying serum for thyroid stimulating hormone, luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone, growth hormone, prolactin, or a subcombination of these hormones. Other endocrine abnormalities, such as the multiple endocrine neoplasias may also be easily detected by devices of the present invention. Persons of skill will appreciate many

other combinations of analytes that may be simultaneously detected by devices of the present invention.

Devices for a plurality of determinations of the presence or amount of one or more analytes in a series of samples are also provided. These devices are particularly useful for home monitoring of physiological conditions, such as infertile periods during a female's menstrual cycle.

Generally, devices of this type comprise a matrix defining a flow path; a plurality of sample receiving zones on the matrix; a plurality of assay strips located downstream from the sample receiving zones, wherein each assay strip is in fluid connection with only one sample receiving zone and comprises a labelling zone having a means for labelling the analyte and a capture zone located downstream from the labelling zone. Application of a sample to the sample receiving zone results in specific binding of the analyte in the sample to labelling means on the assay strips and accumulation of labelled analyte in the capture zones.

The assay strips of this embodiment of the present invention are constructed and function as described above. Each assay strip is in fluid contact with only one sample receiving zone. Different samples are applied to different sample receiving zones. Thus, the same analytes are measured by each assay. Samples may be serially tested in this manner to detect changes in physiological states or disease conditions.

The devices of the present invention may include housings that contain the matrixes. The housings are typically constructed of plastic, but other materials such as vinyls, nylons, or the like may be used. The housings have a top plate covering the assay surface of the matrixes. The top plate has a means for transmitting the sample to the matrix. Generally, the transmitting means is a sample well that is an opening in the top plate. The sample well is located over the sample receiving zone. The top plate also has a means for observing accumulation of label in the capture zone following application of the sample. Typically, the observation means

are result windows in the top plates. The result windows are located over the capture zones of the assay strips on the matrix. Generally, the result windows are open, but the windows may be covered by a transparent covering such as glass or plastic. The housings may also contain dividers between the assay strips to inhibit flow of sample between assay strips.

The top plates may have a variety of configurations. In devices for simultaneous detection of several analytes, a single sample well and a plurality of result windows (one for each assay strip) are present. If the matrix has control test strips, result windows are located over the capture zones of the control test strips. A control sample well is located over the second sample receiving zone, when present. Individual sample wells for each assay strip are also generally present in devices for serially detecting an analyte in samples.

The present invention also provides methods for simultaneously determining the presence or amount of a plurality of analytes in a sample. Generally, the methods comprise applying the sample to a device as described above and observing the accumulation of visible label within the capture zone as a result of analyte present in the sample specifically binding to the labelling complex in the labelling means and the resulting analyte-labelling complexes flowing into and being retained in the capture zone.

Methods for serial detection of an analyte in samples are also provided. Individual samples are applied to the sample receiving zones and accumulation of label is observed through the result windows. Samples may be serially assayed to detect changes in the level of the assay and analyte.

Referring now to Fig. 1, an assay strip 10 is illustrated. This assay strip 10 has three components, a labelling zone 12, a capture zone 14, and an absorbent zone 16. The labelling means is located in the labelling zone 12. The second analyte-binding substance is located in the capture

zone 14. Positive and negative test strips may have similar construction differing from the assay strips as described above. Fig. 2 shows one embodiment of an assay strip 10 as in Fig. 1. The second analyte-binding substance 18 is immobilized in the capture zone 14 as a "+", although immobilization in any shape, including a line, is appropriate. A procedural control line 20 is also present in the capture zone 14. The procedural control line 20 is located downstream from the second analyte-binding substance 18. Therefore, if the procedural control line 20 is exposed to and captures visible label in the sample fluid, the second analyte-binding substance 18 will have also been exposed to the sample fluid.

Fig. 3 illustrates one embodiment of a device 26 for the simultaneous detection of seven different analytes in a sample. The device 26 has a fan shaped matrix 24. Seven assay strips 10 are located on the matrix 24. Each assay strip 10 has a labelling zone 12, a capture zone 14, and an absorbent zone 16. Generally, the labelling means in the labelling zones 12 and the second analyte-binding substances in the capture zones 14 will be different in each assay strip 10. Each assay strip 10 is in fluid communication and located downstream from a sample receiving zone 22. The assay strips 10 extend radially from the sample receiving zone 22. A sample applied to the sample receiving zone 22 will flow into each assay strip 10 resulting in detection of each target analyte.

Fig. 4 illustrates a housing top plate 40 for a device that simultaneously detects different analytes in a sample. The top plate 40 has a sample well 42 and several result windows 44. When the top plate 40 is positioned over the matrix of the device, the sample well 42 is located directly over the sample receiving zone on the matrix. The result windows 44 are located directly over the capture zones of the assay strips on the matrix. The sample may be applied to the sample receiving zone through the sample well 42 and label accumulation in the capture zones may be observed through the result windows 44.

Another embodiment of the present invention is shown in Fig. 5. This device 28 provides a means for seven serial determinations of an analyte. Seven assay strips 10 are present on a matrix 24. Each assay strip 10 has an individual sample receiving zone 30 that does not fluidly communicate with any other assay strip 10. The assay strips 10 contain identical labelling means and second analyte-binding substances. Seven different samples may be analyzed by the device 28.

A housing top plate 50 for a device for serial detection of an analyte in different samples is illustrated in Fig. 6. The top plate 50 has seven sample wells 52 and seven result windows 44. Each assay strip on the underlying matrix is associated with only one sample well 52 and only one result window 44. Individual samples may be applied to the assay strips through the sample wells 52 and the results discerned through the result windows 44.

Fig. 7 illustrates another embodiment of the present invention having a positive control strip 32 and a negative control strip 34. This embodiment has a matrix 24 containing a first sample receiving zone 38 in fluid contact with five assay strips 10. The matrix 24 also contains a second sample receiving zone 36 in fluid contact with a positive assay strip 32 and a negative assay strip 34. The first sample receiving zone 38 does not fluidly communicate with the positive control strip 32 or the negative control strip 34. The second sample receiving zone 36 does not fluidly communicate with the assay strips 10. A test fluid is applied to the second sample receiving zone 36 to assess the functioning of the device 30 through the positive control strip 32 and the negative control strip 34.

Fig. 8 shows a housing top plate 60 of a device for simultaneous detection of five analytes in a sample. The device has positive and negative control strips that are assessed by application of a control test fluid to the control strips. The sample is applied to the first sample receiving zone through a sample well 64. The sample can flow onto the

assay strips and accumulation of label in assay strip capture zones can be detected through result windows 44. The test fluid is applied to the second sample receiving zone on the matrix through a control sample well 66. Accumulation of label on the positive and negative control strips can be assessed through control result windows 62.

WHAT IS CLAIMED IS:

1. A device for simultaneous detection of a plurality of target analytes in a sample, comprising:

a first matrix defining a flow path;

a first sample receiving zone on the first matrix; and

a plurality of assay strips in fluid contact with the first sample receiving zone and located downstream from the first sample receiving zone, wherein each assay strip detects a different target analyte, which assay strips each comprise a labelling zone having a means for specifically labelling the target analyte detected by the assay strip and a capture zone located downstream from the labelling zone;

whereby application of the sample to the first sample receiving zone results in specific binding of the different target analytes in the sample to the labelling means on the assay strips and accumulation of labelled target analytes in the capture zones.

2. A device as in claim 1, further comprising a plurality of absorbent zones, wherein each absorbent zone is located downstream from at least one capture zone.

3. A device as in claim 1, further comprising:

a positive test strip and a negative test strip, which test strips are located downstream from the first sample receiving zone wherein,

the positive test strip detects a non-target analyte normally present in the sample and comprises a second labelling zone having a second means for labelling the non-target analyte and a second capture zone located downstream from the labelling zone, and

the negative test strip comprises a third capture zone and a third labelling zone having a third labelling means, which third labelling means does not label any analyte in the sample;

whereby application of the sample to the first sample receiving zone results in specific binding of the non-target analyte to the second labelling means and accumulation of labelled non-target analyte in the second capture zone, but no specific accumulation of the third labelling means in the third capture zone.

4. A device as in claim 1, further comprising:

a second matrix defining a second flow path;

a second sample receiving zone for receiving a test fluid on the second matrix;

a positive test strip and a negative test strip, which test strips are located downstream from the second sample receiving zone wherein,

the positive test strip detects a test analyte in the test fluid analyte and comprises a second labelling zone having a second means for labelling the test analyte and a second capture zone located downstream from the labelling zone, and

the negative test strip comprises a third capture zone and a third labelling zone having a third labelling means, which third labelling means does not label any analyte in the test fluid;

whereby application of the test fluid to the second sample receiving zone results in specific binding of the test analyte to the second labelling means and accumulation of labelled test analyte in the second capture zone, but no specific accumulation of the third labelling means in the third capture zone.

5. The device of claim 1, wherein each assay strip further comprises a positive procedural control line.

6. The device of claim 1, wherein each of the assay strips extends radially from the first sample receiving zone.

7. The device of claim 1, wherein the assay strips detect antibodies to toxoplasma gondii, rubella virus, human cytomegalovirus, herpes simplex virus, and treponema pallidum.

8. The device of claim 1, wherein the assay strips detect allergen-specific IgE.

9. The device of claim 1, wherein each assay strip for detecting a target analyte comprises:

a matrix defining an axial flow path, said matrix having a labelling zone with a means for labelling the target analyte having a labelling complex comprising a visible label bound to a first analyte-binding substance, a capture zone located downstream from the labelling zone, and an absorbent zone located downstream from the capture zone; and

a second analyte-binding substance immobilized in the capture zone.

10. A device as in claim 1, further comprising a housing, which housing contains the matrix and comprises one result window for each assay strip and one sample well.

11. A device for a plurality of determinations of the presence or amount of an analyte in a sample, comprising:

a matrix defining a flow path;

a plurality of sample receiving zones on the matrix;

a plurality of assay strips located downstream from the sample receiving zones, wherein each assay strip is in fluid contact with only one sample receiving zone and comprises a labelling zone having a means for labelling the analyte and a capture zone located downstream from the labelling zone;

whereby application of the sample to one of the sample receiving zones results in specific binding of the analyte in the sample to the labelling means on the assay strip and accumulation of labelled analyte in the capture zone.

12. A device as in claim 11, further comprising a plurality of positive test strips and a plurality of negative test strips, wherein each sample receiving zone is in fluid contact with one positive test strip and one negative test strip.

13. A device as in claim 11, wherein the assay strips detect the amount of luteinizing hormone in a sample.

14. A device as in claim 13, wherein the device has seven assay strips.

15. The device as in claim 11, further comprising a plurality of absorbent zones located downstream from the capture zones, wherein each capture zone fluidly contacts with only one capture zone.

16. A device as in claim 11, further comprising a housing, which housing contains the matrix and comprises one sample well for each assay strip and one result window for each assay strip.

17. A device as in claim 11, further comprising a plurality of second assay strips, wherein each second assay strip is in fluid contact with a different sample receiving zone and the second assay strips are located downstream from the sample receiving zones.

18. A device as in claim 17, wherein the first assay strips detect luteinizing hormone and the second assay strips detect progesterone or a progesterone metabolite.

19. A method for simultaneously determining the presence or amount of a plurality of analytes in a sample, comprising:

a. applying the sample to a sample receiving zone on a matrix having a flow path to a plurality of capture zones located downstream from the sample receiving zone,

wherein the sample between the sample receiving zone and each capture zone flows through a plurality of means for labelling the analytes, each labelling means containing a labelling complex comprising a visible label bound to a first selected analyte-binding substance, and

a second selected analyte-binding substance is immobilized in the capture zone; and

b. observing the accumulation of visible label within the capture zone as a result of analyte present in the sample specifically binding to the labelling complex in the labelling means and the resulting analyte-labelling complexes flowing into and being captured within the capture zone.

20. A method as in claim 19, wherein the analytes are antibodies to toxoplasma gondii, rubella virus, human cytomegalovirus, herpes simplex virus, and treponema pallidum.

21. A method as in claim 19, wherein the sample also flows onto a positive test strip and a negative test strip.

22. A method as in claim 19, further comprising

applying a liquid to a second sample receiving zone, wherein the second sample receiving zone fluidly contacts a positive test strip and a negative test strip; and

observing the positive test strip and the negative test strip to detect accumulation of a label.

23. A method for serial determinations of the presence or amount of an analyte in a series of biological samples, comprising:

a. applying a biological sample to a sample receiving zone in a device comprising a matrix defining a flow path, a plurality of sample receiving zones on the matrix, a plurality of assay strips located downstream from the sample receiving zones, wherein each assay strip is in fluid contact with only one sample receiving zone and each assay strip comprises a labelling zone having a means for labelling the analyte and a capture zone located downstream from the labelling zone;

b. observing the capture zone in fluid contact with the sample receiving zone onto which the sample was applied to detect the presence or amount of the analyte in the sample; and

c. repeating steps a and b in different sample receiving zones for each sample.

24. A method as in claim 22, wherein the analyte is luteinizing hormone, progesterone, or a progesterone metabolite.

25. A kit for the simultaneous detection of a plurality of analytes, comprising:

a device as in claim 1; and

a means for quantifying the amount of label retained in the capture zone.

26. A kit as in claim 25, further comprising a test fluid.

27. A kit as in claim 25, wherein the quantifying means is a reflectance analyzer.

28. A kit as in claim 25, wherein the quantifying means is a color key.

29. A kit for serial determinations of the presence or amount of an analyte in a series of biological samples, comprising:

a device as in claims 11;

vials for collection of the samples; and

a color key.

30. A kit as in claim 29, wherein the biological sample is urine and the analyte is luteinizing hormone, progesterone, or a progesterone metabolite.

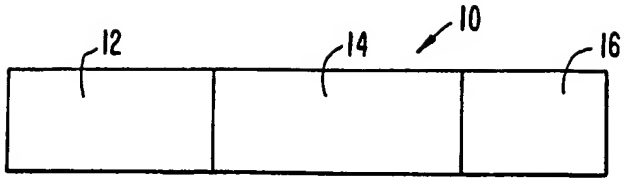

FIG. 1.

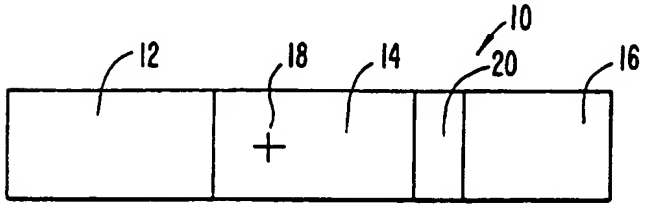

FIG. 2.

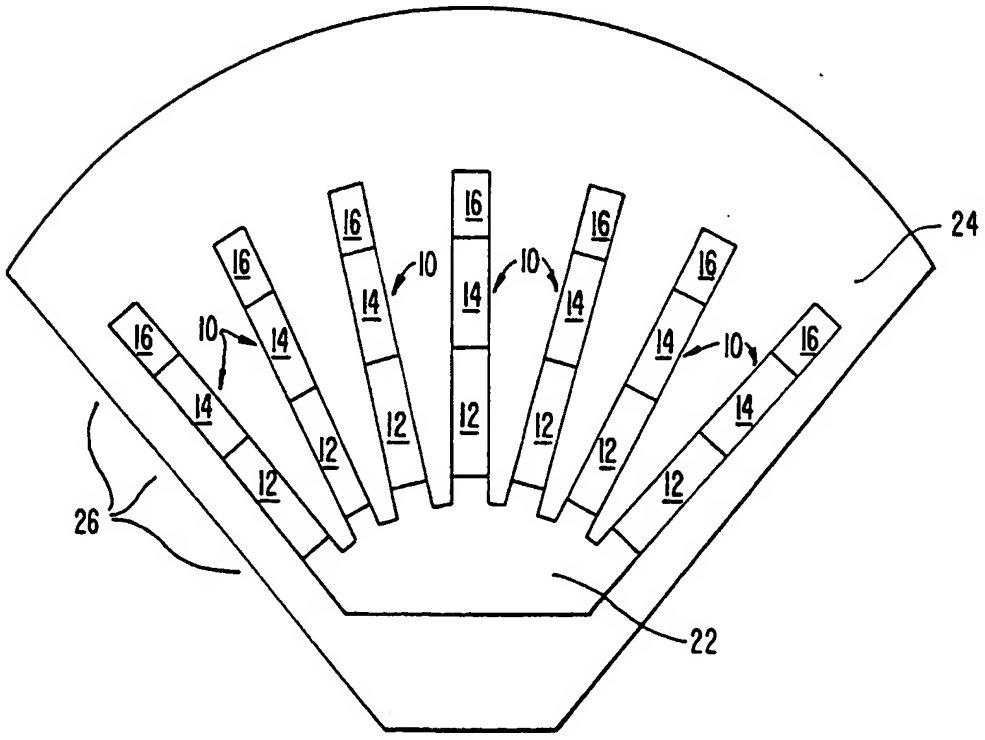

FIG. 3.

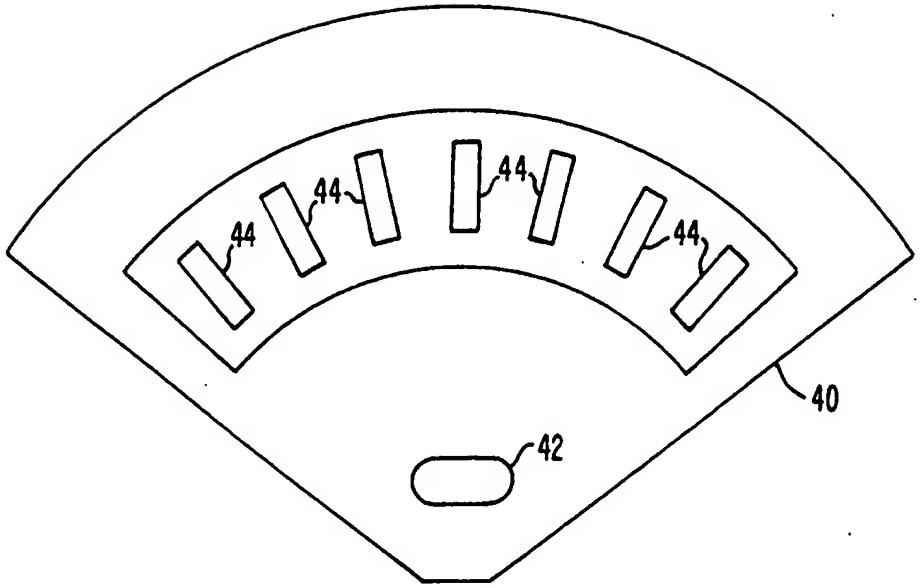

FIG. 4.

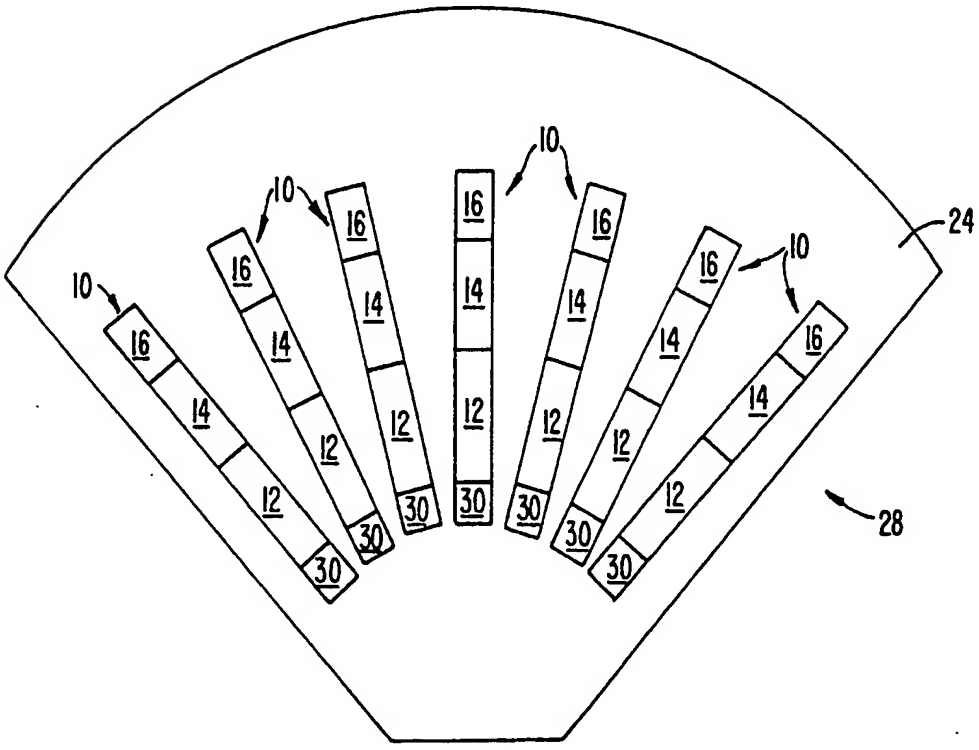

FIG. 5.

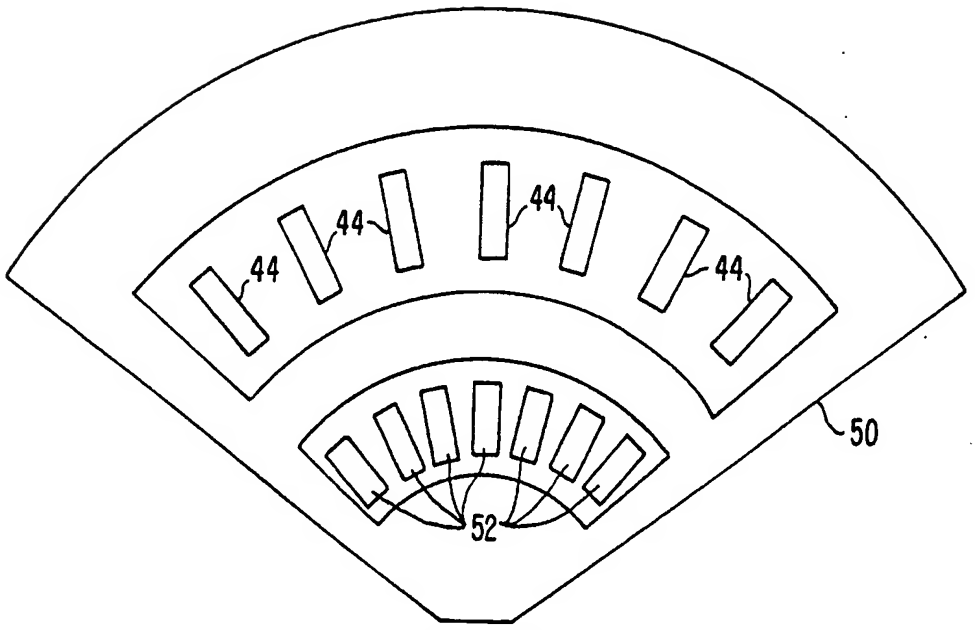

FIG. 6.

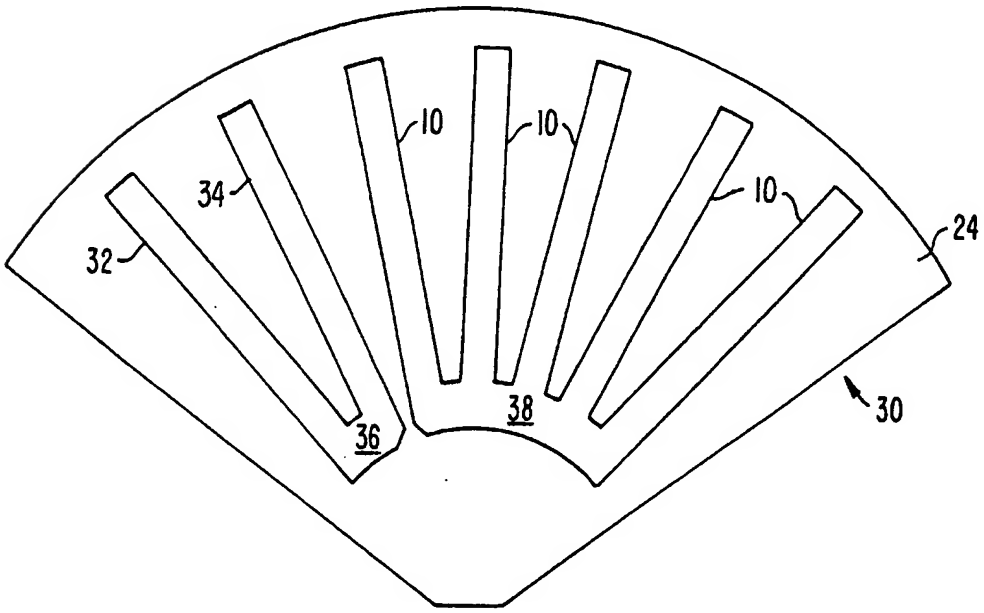

FIG. 7.

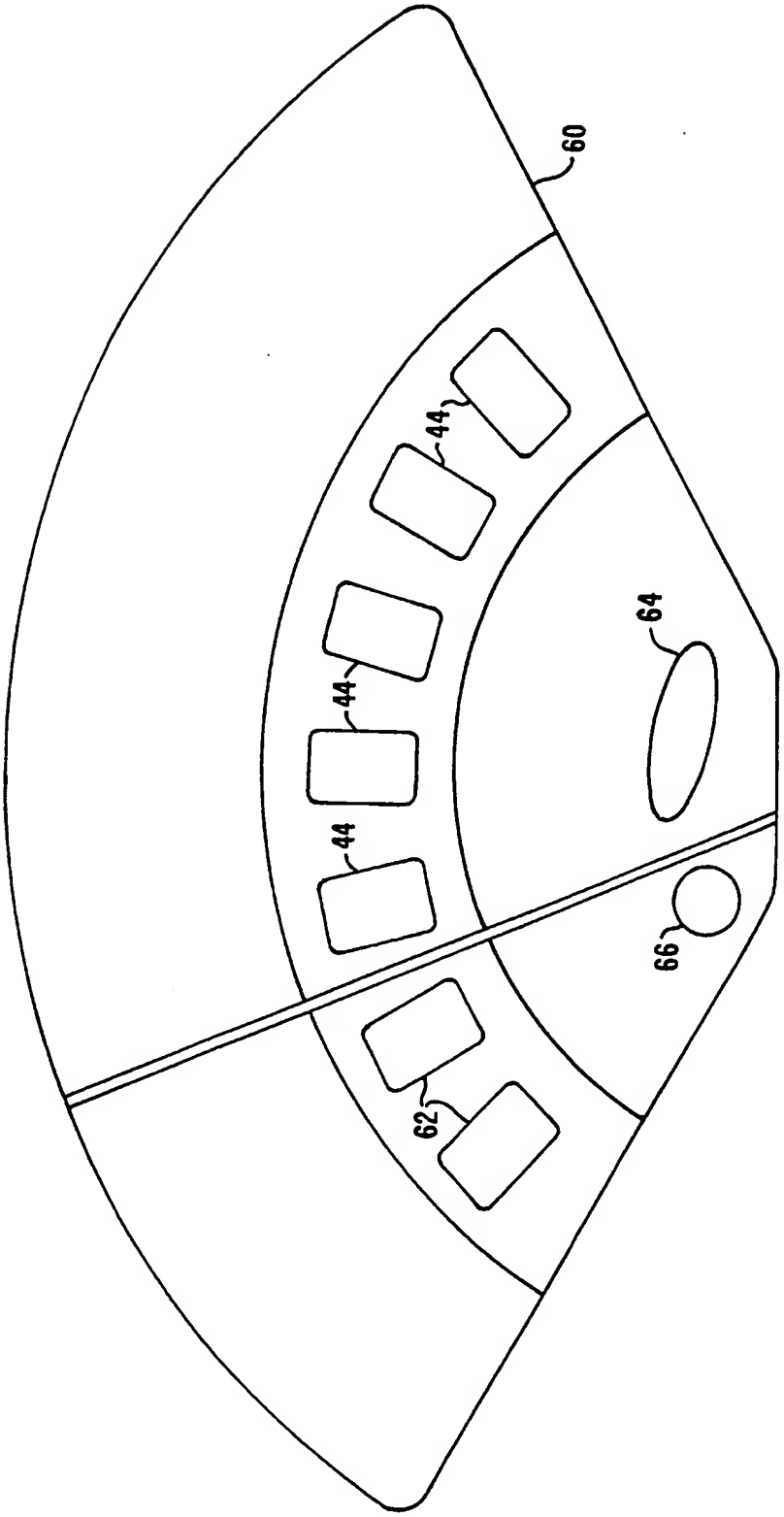

FIG. 8.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03488

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : G01N 33/558
US CL : 435/5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB, 2,239,313 (KANG ET AL.) 26 June 1991, see entire document.	1
Y,P	US, A, 5,238,652 (SUN ET AL.) 24 August 1993, see entire document.	1-10,19-22,24-28
Y	GB, 2,204,398 (MAY ET AL.) 09 November 1988, see entire document.	1-10,19-22,24-28
Y	US, A, 4,968,633 (MARCUCCI) 06 November 1990, see entire document.	8
Y	US, A, 4,877,580 (ARONOWITZ ET AL.) 31 October 1989, see entire document.	28
Y	US, A, 4,904,605 (O'BRIEN ET AL.) 27 February 1990, see entire document.	28

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 MAY 1994	JUN 14 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER CHIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03488

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US, A, 5,240,844 (WIE ET AL.) 31 August 1993, see entire document.	3,4,21,22
Y	Maggio et al, "Enzyme-Immunoassay", published 1980 by CRC Press (Florida), pages 61,171-172, and 190-191, see entire document.	1-10,19-22, 24-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03488

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 19-22, and 24-28

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03488

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/55, 56, 57, 58, 61; 435/4, 5, 7.22, 7.36, 7.9, 7.92, 7.94, 805, 970, 973, 975; 436/169, 510, 511, 513, 514, 518, 528, 530, 531, 805, 970, 973, 975

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-10, 19-22, and 24-28, drawn to a device, method of assay, and kit, classified in Class 435, subclass 5.

II. Claims 11-18, 23, and 29-30, drawn to a device, method of assay, and kit, classified in Class 435, subclass 5.

The claims of Groups I and II are directed to separate and distinct devices and methods of assay which utilize the device included in each of the groups, which are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to provide a single general inventive concept. Note that PCT Rules 13.1 and 13.2 do not provide for multiple distinct devices and methods within a single application.